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ORIGINAL ARTICLE

Anti-*Saccharomyces cerevisiae* antibodies in patients with systemic lupus erythematosus

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Abstract Anti-*Saccharomyces cerevisiae* antibodies (ASCA) had been known to be specific for Crohn's disease but it has been found in many other autoimmune diseases like systemic lupus erythematosus (SLE). Furthermore, cross-reactive epitopes on β2-glycoprotein I (β2GPI) and *Saccharomyces cerevisiae* were found in SLE patients. The aims of this study were to evaluate the frequency of ASCA in patients with SLE and to compare it with that of anti-β2GPI antibodies (aβ2GPI). Sera of 116 patients with SLE were analyzed in this retrospective study. All patients fulfilled at least 4 criteria of the 1997 American College of Rheumatology updated criteria for the classification of SLE. Sera of 160 blood donors were included as normal controls. ASCA IgA and IgG and aβ2GPI antibodies were determined by enzyme-linked immunosorbent assays. The frequency of ASCA (IgG and/or IgA) was significantly higher in SLE patients than in control group (31.9 vs. 3.7 %, $p < 10^{-6}$). ASCA IgG and ASCA IgA were more frequent in SLE patients than in control group (29.3 vs. 3.1 %, $p < 10^{-6}$ and 12.1 vs. 0.6 %, $p = 10^{-4}$, respectively). The mean level of ASCA IgG was higher than that of ASCA IgA (9.5 vs. 6.4 U/ml) but the difference was not statistically significant. The frequencies of aβ2GPI (IgG and/or IgA) and aβ2GPI IgA were significantly higher than those of ASCA (IgG and/or IgA) and ASCA IgA (54.3 vs. 31.9 %, $p = 5 \times 10^{-4}$ and 50.9 vs. 12.1 %, $p < 10^{-6}$, respectively). Increased ASCA IgG was observed in patients with SLE, suggesting a role of environmental stimuli in its pathogenesis.

Keywords Anti-β2GPI antibodies · Anti-*Saccharomyces cerevisiae* antibodies · Systemic lupus erythematosus · Tunisia

Introduction

Antibodies against *Saccharomyces cerevisiae* (ASCA), a yeast commonly used in the food industry, were considered as a serological marker for Crohn's disease, a chronic inflammatory disorder of the intestine [1, 2]. It has also been shown that ASCA had a high predictive value for inflammatory bowel disease [3]. Additionally, increased levels of ASCA had been found in patients with Behcet's disease [4], spondyloarthritis [5], celiac disease [6, 7], intestinal tuberculosis [8], primary biliary cirrhosis [9, 10], autoimmune hepatitis [11], type 1 diabetes [12], and autoimmune thyroid disease [13].

Furthermore, Dai et al. [14] described ASCA in systemic lupus erythematosus (SLE), and Krause et al. [15] described ASCA in primary antiphospholipid syndrome (APLS). Moreover, Krause et al. [15] demonstrated cross-reactive epitopes on β2-glycoprotein I (β2GPI) and *Saccharomyces cerevisiae*. Since anti-β2GPI antibodies (aβ2GPI) are autoantibodies that we found not only in APLS but also in SLE, so the aims of our study were to determine the frequency of ASCA and to compare it with that of aβ2GPI in a large series of Tunisian patients with SLE.

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Materials and methods

Patients

One hundred and sixteen SLE patients were included in this retrospective study (103 females and 13 males, median age: 31 years; age range: 16–78 years). Sera from patients included in this study were selected retrospectively via the database of our immunology laboratory. Sera were collected between 1994 and 2009 from four hospitals in the center of Tunisia. All SLE patients fulfilled at least 4 criteria of the 1997 American College of Rheumatology (ACR) updated criteria for the classification of SLE [16]. Sera of 160 blood donors (128 females and 32 males, mean age: 21 years 4 months) were included as normal controls. All sera were stored at −80 °C until use. The study was approved by local ethics committee, and all patients and/or their parents gave their informed consent.

Methods

Anti-*Saccharomyces cerevisiae antibodies*

ASCA IgA and IgG were detected by a commercially available ELISA kit (Orgentec®, Mainz, Germany). The antigen consisted of highly purified mannan from *Saccharomyces cerevisiae*. Results were expressed as arbitrary units with a cut-off for positivity of 10 U/ml following the manufacturer's instructions.

Anti-β2-glycoprotein I

IgG and IgA aβ2GPI antibodies were determined with a commercial ELISA (Orgentec®) using a purified human β2-glycoprotein I. Results were expressed as arbitrary units with a cut-off for positivity of 8 U/ml following the manufacturer's instructions.

Statistical analysis

The comparison of frequencies was made using chi-square or Fisher's exact test. A p value less than 0.05 was considered significant.

Results

Frequency of ASCA in SLE patients and in the control group

Compared to the control group, SLE patients had a significantly higher frequency of ASCA (IgG and/or IgA) (31.9 vs. 3.7 %, $p < 10^{-6}$), ASCA IgG (29.3 vs. 3.1 %, $p < 10^{-6}$) and ASCA IgA (12.1 vs. 0.6 %, $p = 10^{-4}$) (Table 1).

As shown in Fig. 1, the mean levels of ASCA IgG and IgA were also significantly higher in SLE patients than in the control group (9.5 vs. 2.3 U/ml, $p < 10^{-6}$; 6.4 vs. 3.4 U/ml, $p = 0.001$, respectively).

Frequency of ASCA according to the gender

In SLE patients, the frequency of ASCA IgG was 30.1 % in females and 23.1 % in males. ASCA IgA was present in 23.1 % in males and 10.7 % in females (Table 1).

Comparison between ASCA IgA and ASCA IgG

ASCA IgG was more frequent than ASCA IgA in all SLE patients (29.3 vs. 12.1%; $p = 10^{-3}$) and in females (30.1 vs. 10.7 %; $p = 5 \times 10^{-4}$) (Table 1).

ASCA IgG and ASCA IgA levels were significantly higher in SLE patients than in control group (9.5 ± 13.7 vs. 2.3 ± 2.8, $p < 10^{-6}$, 6.4 ± 11.0 vs. 3.4 ± 1.7, $p = 0.001$, respectively). In SLE patients, the mean titer of ASCA IgG was increased compared to ASCA IgA but reaching a borderline significance (9.5 ± 13.7 vs. 6.4 ± 11.0, $p = 0.06$) (Fig. 1).

Table 1 Comparison of the frequency of ASCA in SLE patients and in the control group and in females and in males

	SLE patients ($n = 116$)	Control group ($n = 160$)	p	SLE females ($n = 103$)	SLE males ($n = 13$)	p
ASCA IgG or IgA	31.9 % (37/116)	3.7 % (6/160)	$<10^{-6}$	33 % (34/103)	23.1 % (3/13)	NS
ASCA IgG and IgA	9.5 % (11/116)	0 %	5×10^{-5}	7.7 % (8/103)	23.1 % (3/13)	NS
ASCA IgG	29.3 %* (34/116)	3.1 % (5/160)	$<10^{-6}$	30.1 %** (31/103)	23.1 % (3/13)	NS
ASCA IgA	12.1 %* (14/116)	0.6 % (1/160)	10^{-4}	10.7 %** (11/103)	23.1 % (3/13)	NS

ASCA anti-*Saccharomyces cerevisiae* antibodies, *SLE* systemic lupus erythematosus

* Comparison between ASCA IgG and ASCA IgA in SLE patients ($p = 10^{-3}$)

** Comparison between ASCA IgG and ASCA IgA in female patients with SLE ($p = 5 \times 10^{-4}$)

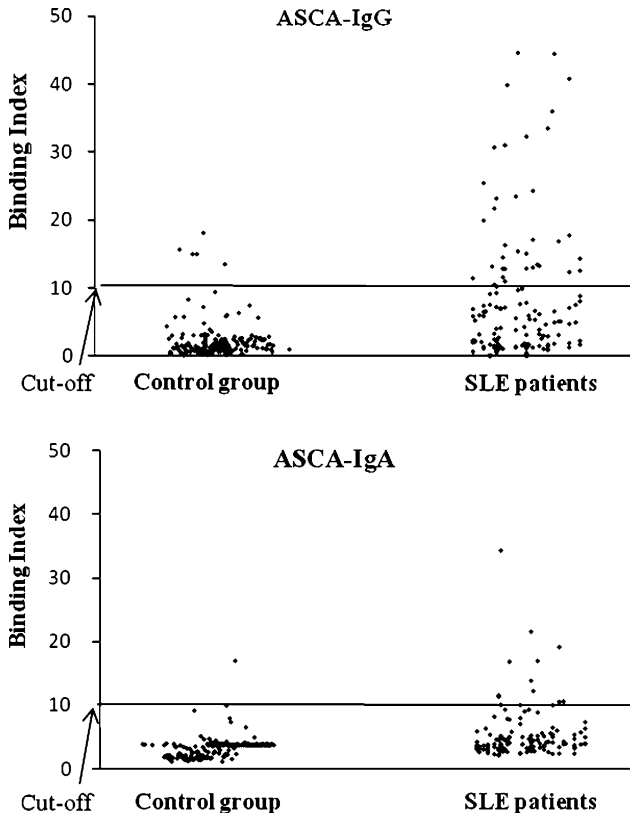

Fig. 1 Comparison of ASCA IgG and IgA levels in SLE patients and healthy subjects. The mean level of ASCA IgG was significantly higher in SLE patients than in the control group (9.5 vs. 2.3 U/ml, $p < 10^{-6}$). The mean level of ASCA IgA was significantly higher in SLE patients than in the control group (6.4 vs. 3.4 U/ml, $p = 0.001$)

Frequency of aβ2GP1 in SLE patients

In SLE patients, the frequency of aβ2GPI (IgG or IgA) was 54.3 %; it was 55.3 % in females and 0 % in males. aβ2GP1 IgG were 21.4 % in females and 7.7 % in males. aβ2GP1 IgA were slightly higher in females than in males (52.4 and 38.5 %, respectively) (Table 2).

aβ2GP1 IgA was more frequent than aβ2GP1 IgG in all SLE patients (50.9 vs. 19.8 %; $p < 10^{-6}$) and in females (52.4 vs. 21.4 %; $p = 7 \times 10^{-6}$) (Table 2).

Table 3 Frequency of ASCA and aβ2GP1

	aβ2GP1	ASCA	p
IgG or IgA	54.3 % (63/116)	31.9 % (37/116)	5×10^{-4}
IgG and IgA	16.4 % (19/116)	9.5 % (11/116)	NS
IgG	19.8 % (23/116)	29.3 % (34/116)	NS
IgA	50.9 % (59/116)	12.1 % (14/116)	$<10^{-6}$

ASCA anti-*Saccharomyces cerevisiae* antibodies, *aβ2GPI* anti-β2-glycoprotein I antibodies

Comparison between ASCA and aβ2GPI

The frequencies of aβ2GPI IgG and/or IgA and aβ2GPI IgA were significantly higher than those of ASCA IgG and/or IgA and ASCA IgA (54.3 vs. 31.9 %, 5×10^{-4}, 50.9 vs. 12.1 %, $p < 10^{-6}$, respectively) (Table 3).

Discussion

The frequency of ASCA IgG found in our SLE patients was lower than that found by Dai et al. [14] (29.3 and 57.5 %, respectively). In our study, both ASCA IgG and ASCA IgA were significantly higher in SLE patients than in the control group (29.3 vs. 3.1 %, 12.1 vs. 3.1 %, respectively). However, in the study of Dai et al. [14], only ASCA IgG were significantly higher in SLE patients than in the control group (57.5 vs. 8.5 %). This discrepancy could be explained by the fact that Dai et al. established their own ELISA systems, whereas we used a commercially available ELISA. In addition, it may be due to the differences between the number of SLE patients included in the study. In fact, we included 118 SLE patients compared to 40 in the study of Dai et al. [14].

The high frequency of ASCA could be explained by the cross-reactivity between *β2GPI and Saccharomyces cerevisiae*. The frequency of a*β2GPI* (IgG or IgA) was 54.3 % in our SLE patients; it ranges from 12 to 44 % in the literature [17]. In our SLE patients, the frequency of a*β2GPI* was significantly higher than that of ASCA (54.3 vs. 31.9 %,

Table 2 Frequency of aβ2GP1 according to the gender

	All SLE patients ($n = 116$)	Females ($n = 103$)	Males ($n = 13$)	p
aβ2GP1 IgG or IgA	54.3 % (63/116)	55.3 % (57/103)	0 % (0/13)	5×10^{-4}
aβ2GP1 IgG and IgA	16.4 % (19/116)	18.4 % (19/103)	0 % (0/13)	NS
aβ2GP1 IgG	19.8 %* (23/116)	21.4 %** (22/103)	7.7 % (1/13)	NS
aβ2GP1 IgA	50.9 %* (59/116)	52.4 %** (54/103)	38.5 % (5/13)	NS

aβ2GPI anti-β2-glycoprotein I antibodies

* Comparison between aβ2GP1 IgG and aβ2GP1 IgA in SLE patients ($p < 10^{-6}$)

** Comparison between aβ2GP1 IgG and aβ2GP1 IgA in SLE female patients ($p = 7 \times 10^{-6}$)

$p = 5 \times 10^{-4}$). Even in the study of Krause et al., the rate of aβ2GPI (IgG and/or IgM) (68.4 %) was higher than that of ASCA (20 %). Indeed, only a subpopulation of aβ2GPI is specific to the glycosylated site of the β2GPI molecule that cross-reacts with phosphopeptidomannan [15].

Moreover, in the other autoimmune disorders in which ASCA have been described, such as Crohn's disease [1], celiac disease [7], Behcet's syndrome [4], and primary biliary cirrhosis [9], there is an increased intestinal permeability [18–20]. Intestinal permeability in SLE was analyzed previously [19, 21]. In the study of Wang [21], no significant difference was demonstrated about intestinal permeability between SLE patients and the controls and the authors concluded for a possibility of not validity of the Cr-EDTA permeability test. However, using the same test, Fresko et al. found that intestinal permeability was higher in SLE patients than in healthy controls [21]. On the other hand, it has been demonstrated that occasional increases in intestinal permeability in SLE were related to protein losing enteropathy (PLE). It is a glycoprotein synthesized by the liver, with a molecular weight similar that of albumin. When leaked into gastrointestinal lumen, it is digested minimally by intestinal proteases and then excreted in the stool [22]. Several factors associated with inflammation, such as oxidant stress and exposure to various cytokines, could be related to increased intestinal permeability. In fact, during inflammation, the intestinal barrier can be disrupted, indicated by a decrease in transcellular electrical resistance and an increase in paracellular permeability for tracers of different size [23].

Furthermore, it has been demonstrated that Candida albicans is an immunogen for ASCA markers of Crohn's disease [24]. Overexpression of major ASCA epitopes in *C. albicans* occurs when the yeast infects human tissues. In fact, the susceptibility of SLE patients to cutaneous and oral candidosis has been well documented and has been linked to the ability of *C. albicans* to adhere to mucous membranes [25]. The question arises of whether the high frequency of ASCA found in SLE is related to the increased immunity to *C. albicans*.

In conclusion, patients with SLE had a high frequency of ASCA. Further studies are needed to determine the role of ASCA in the pathogenesis of SLE.

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Conflict of interest None.

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